



Miltenyi Biotec

# autoMACS™ Pro Separator

Short instructions, version 2



# I Before you start

**Tip:** To view the current software version, from the software menu select **Option>User Settings>About>Run**

**Note:** For users wishing to install the the autoMACS Pro Upgrade Kit please refer to section 3.2 of the autoMACS Pro Separator User Manual. Software older than version 2.0 cannot perform autolabeling. If in doubt contact your local sales representative or Miltenyi Biotec autoMACS Pro specialist.

## 1) Choose a magnetic labeling and cell separation approach

- Positive selection or depletion.
- Single parameter sorting or MACS® MultiSort Technology.
- Direct or indirect MACS MicroBeads.
- Automated magnetic labeling (autolabeling) or manual magnetic labeling.

## 2) Choose appropriate sample tube rack

- Select the type of tube rack (e.g. Chill 15) according to the desired number of samples, number of cells and sample volume (see table C in section III).
- Cool the tube rack at 2–8 °C for at least 3–4 hours.
- Place MACS Reagent Rack beside the chosen Chill Rack.

## 3) Prepare cell samples

- Prepare single-cell suspensions, remove cell aggregates.
- Avoid excess of dead cells.
- For manual labeling follow labeling instructions in the MACS MicroBeads datasheet.
- For automated magnetic labeling prepare cells in appropriate labeling volume as outlined in the datasheet.

MACS® Product	Strategy	Minimum total labeling volume for first labeling step*	Maximum total labeling volume
Direct MicroBeads**	Positive selection	200 µL (2×10 <sup>7</sup> total cells)	6.5 mL (6.5×10 <sup>8</sup> total cells)
Cell Isolation Kit	Untouched selection	200 µL (4×10 <sup>7</sup> total cells)	6.5 mL (6.5×10 <sup>8</sup> total cells)
MicroBead Kits**	Positive selection, 2-reagent labeling	200 µL (4×10 <sup>7</sup> total cells)	6.5 mL (6.5×10 <sup>8</sup> total cells)

\* For less cells use same volumes. \*\* For target cell frequencies < 5%, removal of the labeling reagent is recommended (i.e. manual magnetic labeling).

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## II How to use the autoMACS Pro Separator

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### 1. Setup and prime the autoMACS Pro Separator

- Attach filled fluid containers at appropriate positions and empty the waste. Attach the fluid sensor cables accordingly.

**Note:** The connectors and cables for the fluid containers are color-coded: blue for Running Buffer, green for Washing Solution, black for Storage Solution, and red for the waste container.

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- Switch on the autoMACS Pro Separator.
- After initialization of the instrument, the touchscreen displays the menu **Status**. Verify that symbols for the bottles (1) and columns (2) are coded green and that the MACS MiniSampler (3) is detected as displayed in Figure 1.



Figure 1 Status menu at startup

**Note:** If column symbol is marked in red install a pair of fresh autoMACS Separation Columns (for instructions see section IV). Symbol for Storage Solution remains grey. Rack detection (3) occurs upon starting the separation. For further information on the **Status** menu, see section III.

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- Select menu tab Separation.
- Select **Wash Now, Rinse** and press **Run**.

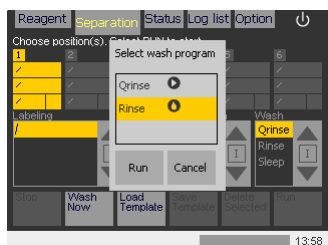


Figure 2 Selecting a wash program "Rinse"

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## 2a. Define a reagent rack using autolabeling

- Select menu **Reagent**.
- Highlight reagent position (e.g. position R1).
- Select **Read Reagent**. The barcode reader will start blinking.
- Scan 2D code from the reagent vial — optimal reading distance is 0.5–2.5 cm.
- Place vial on the corresponding position.
- Repeat the procedure for other reagent vials.

**Note:** Positions R1–R4 on the display correspond to vial positions on the reagent rack. Rack templates can be saved under **Save Template** and reloaded under **Load Template**. Information on scanned reagents is displayed in the **Info** box. If a kit contains more than one vial all reagents must be scanned.



Figure 3 Reagent “CD4 MicroBeads, human” (2) was assigned to position R1 (1) on the MACS Reagent Rack 4

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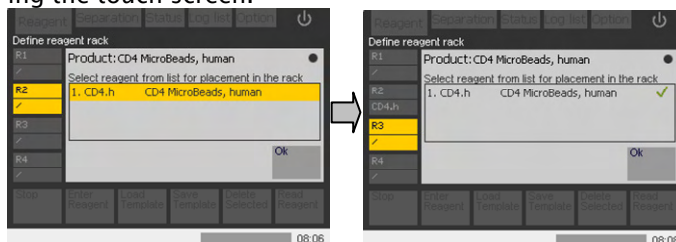
## 2b. Define a reagent rack manually

- Select menu **Reagent**.
- Select reagent position.
- Select **Enter Reagent** from the lower navigation bar.
- Enter the reagent-specific product number



Figure 4 Manually entering reagent information using the “Enter Reagent” command

- Select **Ok**. If a correct number is inserted the software will immediately recognize the reagent or kit. To confirm, select the reagent from the list by using the touch screen.



**Figure 5** CD4 MicroBeads, human (product number 130-045-101) was manually entered

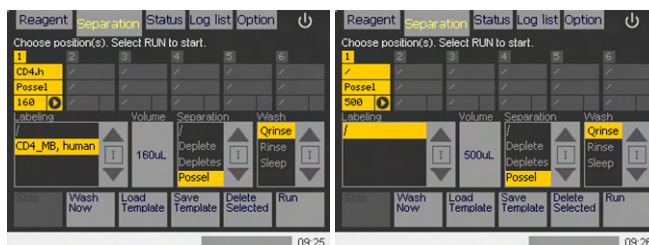
- Place vial on the corresponding position.
- Repeat the procedure for other reagent vials.

### 3. Define autoMACS Pro separation template

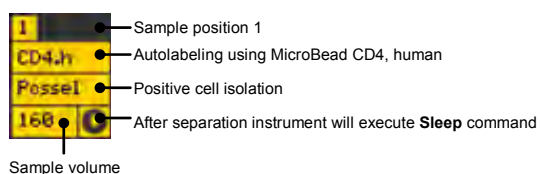
- Select menu **Separation**.
- Setup the sample position(s).
  - Autolabeling: Use the **Labeling** submenu to select a reagent for labeling. Use the **Separation** and **Wash** submenus to select the desired separation and wash programs for each sample.
- Manual labeling: Ensure that no reagent is selected under the **Labeling** submenu (i.e. "/" is highlighted). Use the **Separation** and **Wash** submenus to select the desired separation and wash programs for each sample.
- Enter volume for each sample by touching the volume definition button and selecting the volume in  $\mu\text{L}$ .
- All selected parameters are now displayed for each sample.
  - Tube rack position
  - MACS Reagent Kit (short name)
  - Sample volume
  - Separation and wash programs

**Note:** Positions on the template correspond to positions in the tube rack: Chill 5 (positions 1-6), Chill 15 (positions 1-5), Chill 50 (positions 1-3). Templates can be saved under **Save Template** and reloaded under **Load Template**.





**Figure 6** Performing MicroBead CD4<sup>+</sup> cell separation with autolabeling (left) and without autolabeling (right). The cell separation (Possel) and wash conditions (Qrinse) for both processes are identical. Disabling autolabeling ('/') influences the initial sample volume. For manual labeling it is recommended to dilute cells to a volume of 500 µl / 10<sup>8</sup> total cells (see corresponding datasheet for further information).



**Figure 7** The selected parameters are displayed on the sample definition template

## 4. Perform cell separation

Dilute single-cell suspension according to the recommendations in the product datasheet.

### 1) Autolabeling:

- Dilute cells in volume required for the first labeling step, e.g.:

MACS Product	Strategy	Reagents	Cell concentration*	Minimal volume
Direct MicroBeads – human – rat – non-human primate	Positive selection or Depletion	1	10 <sup>7</sup> cells per 80 µL	160 µL
Direct MicroBeads – mouse	Positive selection or Depletion	1	10 <sup>7</sup> cells per 90 µL	180 µL
Whole Blood MicroBeads (Chill 50)	Whole blood or bone marrow	1	Original volume	4 mL – 8 mL
Cell Isolation Kits	Untouched selection	2	10 <sup>7</sup> cells per 40 µL	160 µL
Cell Isolation Kits	Untouched	3	10 <sup>7</sup> cells per 30 µL	120 µL

MACS Product	Strategy	Reagents	Cell concentration*	Minimal volume
	selection			
MicroBead Kits	Positive selection or Depletion	2	10 <sup>7</sup> cells per 60 µL	120 µL
* When working with fewer cells than the required minimal volume, resuspend cells in the indicated minimal volume.				



## 2) Manual labeling:

- Dilute cells to a final concentration of 500 µl / 10<sup>8</sup>.
- Place cells (sample tubes) and fraction collection tubes in the appropriate tube rack.

**Note:** Row "A" holds sample tubes; row "B": tube for non-labeled fractions; row "C": tubes for labeled fractions. Racks must be pre-cooled for 3-4 hours. Do not chill below 0 °C.



- Place tube rack onto the MACS MiniSampler.

**Note:** Verify that position 1 is orientated to the left.

-  - Select **Run** to start the cell separation.
-  - Select the **Status** menu to monitor the status of the instrument during operation.

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## 5. Shutdown the autoMACS Pro Separator

-  – Press the shutdown symbol (upper right-hand corner of the display).
  -  – Alternatively, select **Sleep** program as the last washing step.
- Turn off the instrument using the main power switch.

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## III Monitoring the autoMACS Pro Separator

The autoMACS Pro Separator is a sensor-controlled device. This feature facilitates monitoring the instrument status during operation. For details see the tables below. Further information on the status is displayed by touching the symbols on the screen.

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### 1 “Status” menu

#### A. Bottle status

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**Note:** Before you start, make sure that the volume of each solution is sufficient for the defined separation template and that the waste bottle is empty.


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Container	Symbol	Symbol color and user action
Running Buffer		Green: No action required
Washing Solution		Red: Refill container Gray: Connect bottle sensor
Storage Solution (70 % Ethanol)		Gray: No liquid detection; visually check volume
Waste		Green: No action required Red: Empty waste Gray: Connect bottle sensor






## B. Column status

**Note:** The fill level on the symbol is an indicator for the remaining service life of the autoMACS Pro Column.

Symbol	Symbol color and user action
	Green: No action required Red: Exchange column (see section IV)



## C. Rack detection

**Note:** Rack detection occurs after separation is started.

Symbol	Features	
	Chill 5	Up to 6 cell separations, max. $5 \times 10^8$ total cells in 2.5 mL per separation
	Chill 15	Up to 5 cell separations, max. $2.5 \times 10^9$ total cells in 12.5 mL per separation
	Chill 50	Up to 3 cell separations, max. $4 \times 10^9$ total cells in 50 mL per separation

## D. MACS MiniSampler detection

**Note:** Detection occurs as soon as the MACS MiniSampler is connected.

Symbol	Features	
	MACS MiniSampler detected	No action required
	No sampler detected	Connect MACS MiniSampler

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## 2 Bottle illumination

**Tip:** Bottle illumination can be switched ON/OFF by selecting **Option> User Settings> O\_led>** and **Run**

Code	Status	User action
Green	Ready for separation	No action required
Blue	Instrument operating	No action required
Yellow	Not ready for separation	Run wash program ( <b>Rinse</b> or <b>Qrinse</b> )
Red	Error	Check display for details
Purple	Program <b>Sleep</b> is completed	Switch off autoMACS Pro Separator
Blinking	Action required	Check screen for required action

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## IV autoMACS Column exchange

Replace autoMACS Columns every 2 weeks, or after 100 separations, whichever comes first.

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### 1 Select “Col\_ex” program

- Select menu **Option**
- Select **Special** and **Col\_ex**
- Press **Run**
- When prompted, exchange columns as described in section 2

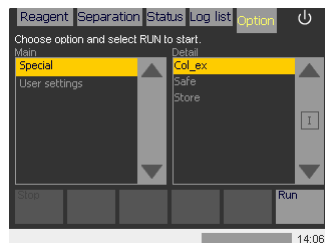


Figure 8 “Col\_ex” program

## 2 Exchange autoMACS Columns

- Open front door and note the positions of the columns (column 1: left; column 2: right). Exchange one column at a time.
- Remove column from slot; unscrew top column connector followed by the bottom column connector as shown in Figure 9.
- Dispose of the expired column.
- Point the bottom of the fresh column towards the autoMACS Pro Separator.
- Insert bottom column connector. Screw in the column by turning it clockwise. Repeat the procedure for the top column connector.

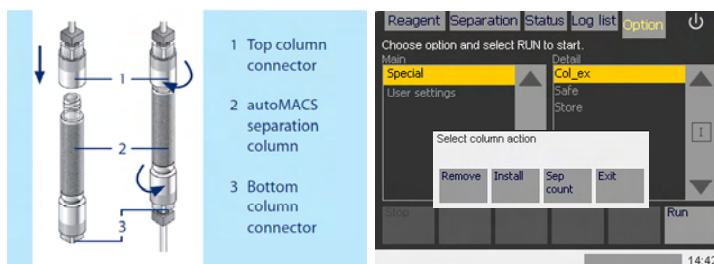


Figure 9 Left: Exchange of the column. Right: Starting the “Col\_ex” program

- Push column into the magnet housing, with the top column connector sitting on the guide in the column slot.
- Repeat installation for the second autoMACS Column.
- After exchange of separation columns, select **Done**. The autoMACS Pro Separator system will be automatically primed with Running Buffer and is then ready for cell separation.

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## V Monitoring the autoMACS Pro Separator

### Rinsing programs

Program	Description	Recommended usage	Duration
Qrinse	Standard short rinse of separation columns and tubing system with Running Buffer	Between separations of frequent cells (> 5 %)	1.5 min
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Between and before separations of rare cells (< 5%)	4 min

### Daily maintenance programs

Program	Description	Recommended usage	Duration
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Prior to first separation	4 min
Sleep	Rinse with Washing Solution followed by filling with Storage Solution	Before switching OFF the autoMACS Pro Separator	5 min

### Periodic maintenance

Program	Description	Recommended usage	Duration
Column exchange	Replacement of separation columns	Every 2 weeks OR after 100 separations, whichever comes first	6 min
Safe	Decontamination procedure with MACS Bleach solution	Every 3–6 months	21 min
Pump syringe	Cleaning of pump syringe (see user manual)	Every 1–3 months	
Store	Rinse with Washing Solution, followed by Storage Solution; replacement of columns and substitutes	Before storing the instrument for a period longer than 2 weeks	

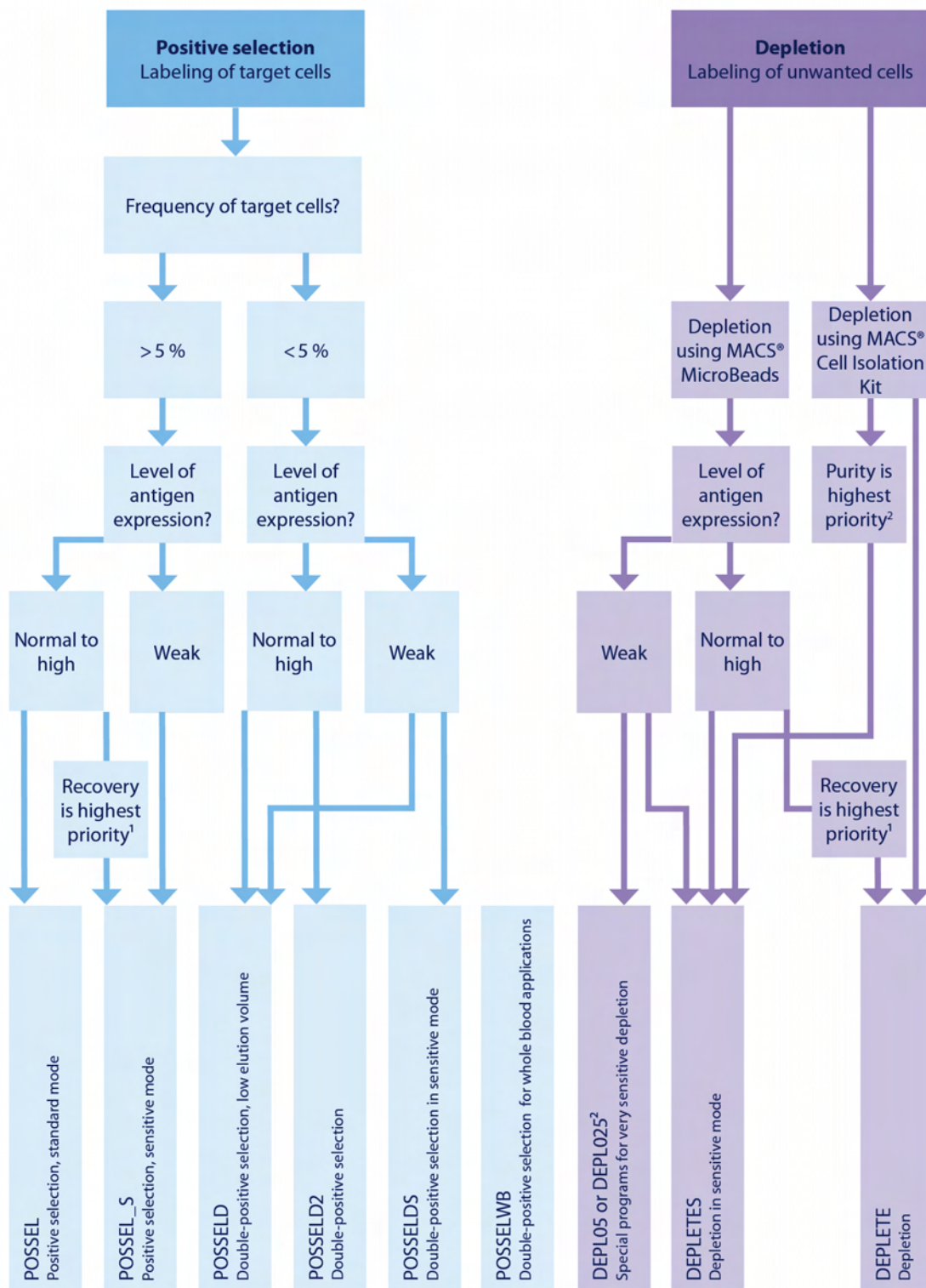
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## VII Solutions

### Solutions required for daily operation

Name	Description
Running Buffer	autoMACS Running Buffer (# 130-091-221)
Washing Solution	autoMACS Pro Washing Solution (# 130-092-987)
Storage Solution	70% v/v ethanol in distilled water, diluted from 100% ethanol (without additive)

## VIII Choosing the optimal program



**Note:** <sup>1</sup>Purity will slightly increase. <sup>2</sup>Recovery will slightly decrease.





Miltenyi Biotec

**Miltenyi Biotec GmbH**  
Friedrich-Ebert-Straße 68  
51429 Bergisch Gladbach  
Germany  
Phone +49 2204 8306-0  
Fax +49 2204 85197  
macs@miltenyibiotec.de

[www.miltenyibiotec.com](http://www.miltenyibiotec.com)

**Miltenyi Biotec Inc.**  
12740 Earhart Avenue  
Auburn, CA 95602  
USA  
Phone 800 FOR MACS,  
+1 530 888 8871  
Fax +1 530 888 8925  
macs@miltenyibiotec.com

**Miltenyi Biotec  
Australia Pty. Ltd.**  
Phone +61 02 8877 7400  
macs@miltenyibiotec.com.au

**Miltenyi Biotec B.V. (Benelux)**  
macs@miltenyibiotec.nl  
**Customer service Netherlands**  
Phone 0800 4020120  
**Customer service Belgium**  
Phone 0800 94016  
**Customer service Luxembourg**  
Phone 800 24971

**Miltenyi Biotec Trading  
(Shanghai) Co., Ltd. (P.R. China)**  
Phone +86 21 6235 1005  
macs@miltenyibiotec.com.cn

**Miltenyi Biotec SAS (France)**  
Phone +33 1 56 98 16 16  
macs@miltenyibiotec.fr

**Miltenyi Biotec S.r.l. (Italy)**  
Phone +39 051 646 0411  
macs@miltenyibiotec.it

**Miltenyi Biotec K.K. (Japan)**  
Phone +81 3 5646 8910  
macs@miltenyibiotec.jp

**Miltenyi Biotec Asia Pacific  
Pte. Ltd. (Singapore)**  
Phone +65 6238 8888  
macs@miltenyibiotec.com.sg

**Miltenyi Biotec S.L. (Spain)**  
Phone +34 91 512 12 90  
macs@miltenyibiotec.es

**Miltenyi Biotec Ltd. (UK)**  
Phone +44 1483 799 800  
macs@miltenyibiotec.co.uk